

AMENDMENTS TO THE CLAIMS

1. (Currently Amended) A probe comprising as operably linked components:

- a) a first pair of nucleic acid sequences consisting of a first object sequence and a first complement sequence, said first object and first complement sequences each independently having about 3 to about 150 nucleotides, being substantially complementary to each other, and forming a first hybridized duplex;
- b) a recognition element conjugated to said first object or first complement sequences through a coupling element, said recognition element specifically interacting with a target agent, said coupling element being essentially the same size as or shorter than the size of said target agent; and
- c) at least one detectable label, said detectable label producing a characteristic signal whose level is a function of the amount of said first hybridized duplex, wherein said recognition element is conjugated through said coupling element to a location inside the first hybridized duplex region of said first object or first complement sequence so that said recognition element is branched out from said first hybridized duplex; and

wherein in the presence of said target agent, said interaction of said target agent with said recognition element alters the amount of said first hybridized duplex compared to that in the absence of said target agent, altering said characteristic signal;

and further wherein said recognition element is selected from the group consisting of chemical ligands, biochemical ligands, antigens, antibodies, antibody fragments, enzymes, substrates of enzymes, inhibitors of enzymes, hormones, antibiotics, narcotics, toxins, polypeptides, proteins, protein fragments, targeting sequences, transit peptides, glycoproteins, lipids, phospholipids, polysaccharides, carbohydrates, and peptide nucleic acids.

2. (Original) The probe of claim 1 further comprising a second pair of competing nucleic acid sequences consisting of a second object sequence and a second complement sequence,

said second object and second complement sequences each independently having about 3 to about 150 nucleotides, being substantially complementary to each other, and forming a second hybridized duplex; and
said first and second object sequences being contained in an object sequence and having an overlapping region consisting of at least one nucleotide,
wherein in the presence of said target agent, said interaction of said target agent with said recognition element causes decrease in the amount of one of said first and second hybridized duplexes and increase in the amount of the other hybridized duplex compared to those in the absence of said target agent, altering said characteristic signal.

3. (Previously Presented) A probe according to claim 2, wherein said recognition element is conjugated to said first object sequence excluding said overlapping region or to said first complement sequence.

4. (Original) A probe according to any of claims 1 and 2, wherein said object and complement sequences are selected from the group consisting of DNA, RNA, PNA, and mixtures of DNA and RNA.

5. (Currently Amended) An affinity probe, comprising as operably linked components:

- a) a first pair of nucleic acid sequences consisting of a first object sequence and a first complement sequence, said first object and first complement sequences each independently having about 3 to about 150 nucleotides, being substantially complementary to each other, and forming a first hybridized duplex;
- b) a probe ligand conjugated to said first object or first complement sequences through a coupling element, said probe ligand specifically interacting with a

receptor agent, said coupling element being essentially the same size or shorter than the size of said receptor agent; and

- c) at least one detectable label, said detectable label producing a characteristic signal whose level of function of the amount of said first hybridized duplex, wherein said probe ligand is conjugated through said coupling element to a location inside the first hybridized duplex region of said first object or first complement sequence so that said probe ligand is branched out from said first hybridized duplex; and
- wherein in the presence of said receptor agent, said interaction of said receptor agent with said probe ligand alters the amount of said first hybridized duplex compared to that in the absence of said receptor agent, altering said characteristic signal; and further wherein said receptor agent specifically binds to said probe ligand;

and further wherein said probe ligand is selected from the group consisting of chemical ligands, biochemical ligands, antigens, antibodies, antibody fragments, enzymes, substrates of enzymes, inhibitors of enzymes, hormones, antibiotics, narcotics, toxins, polypeptides, proteins, protein fragments, targeting sequences, transit peptides, glycoproteins, lipids, phospholipids, polysaccharides, carbohydrates, and peptide nucleic acids.

6. (Previously presented) The affinity probe according to claim 5, wherein the melting temperature of said first hybridized duplex decreases by at least 1°C upon binding of said receptor agent to said probe ligand.

7. (Previously presented) The affinity probe according to claim 5, wherein said probe ligand is covalently linked to said first object or first complement sequence by at least one coupling element.

8. (Previously presented) The affinity probe according to claim 7, wherein said coupling element is selected from the group consisting of chemical bonds, divalent atoms, divalent chemical moieties, and multivalent chemical moieties.

9-57. (Canceled)

58. (Previously presented) A probe according to claim 2, wherein said first hybridized duplex is preferentially formed compared to said second hybridized duplex in the absence of said target agent.

59. (Previously presented) A probe according to claim 58, wherein the melting temperature of said first hybridized duplex is at least 1°C higher than the melting temperature of said second hybridized duplex in the absence of said target agent.

60. (Previously presented) A probe according to claim 58, wherein said second hybridized duplex is preferentially formed compared to said first hybridized duplex in the presence of an excess of said target agent.

61. (Previously presented) A probe according to claim 60, wherein the melting temperature of said first hybridized duplex is at least 1°C lower than the melting temperature of said second hybridized duplex in the presence of an excess of said target agent.

62-81. (Canceled)

82. (Original) A probe according to claim 1, wherein said detectable label is an intercalating dye that can preferentially bind to double-stranded nucleic acids.

83. (Original) A probe according to claim 82, wherein said probe comprises a first molecule comprising said first object sequence and a second molecule comprising said first complement sequence.

84. (Original) A probe according to claim 83, wherein said first or second molecule is immobilized to a support.

85. (Original) A probe according to claim 82, wherein said first object and first complement sequences are covalently linked by a loop moiety.

86. (Original) A probe according to claim 85, wherein said loop moiety connects any of 5' or 3' terminus of said first object sequence to any of 5' or 3' terminus of said first complement sequence.

87. (Original) A probe according to claim 85, wherein said loop moiety is a nucleic acid sequence having about 4 to about 100 nucleotides.

88. (Original) A probe according to claim 87, wherein said first object sequence, said loop moiety, and said first complement sequence are covalently linked in sequence in a 5' to 3' or 3' to 5' direction.

89. (Original) A probe according to claim 85 immobilized to a support.

90. (Original) A probe according to claim 1, wherein said at least one detectable label comprises an interactive label pair comprising a first label moiety conjugated to said first object sequence and a second label moiety conjugated to said first complement sequence, said first and second label moieties interacting when said first hybridized duplex is formed.

91. (Original) A probe according to claim 90, wherein said interactive label pair is a fluorescer and a quencher, wherein the interaction between said interactive label pair causes change in the fluorescence of said probe, said change in the fluorescence being selected from the group consisting of intensity decrease, lifetime decrease, polarization change, and wavelength change.

92-105. (Canceled)

106. (Original) A probe according to claim 1, wherein said probe further comprises a pair of nucleic acid arm sequences consisting of a 5' arm sequence covalently linked to 5' terminus of said first object sequence and a 3' arm sequence covalently linked to 3' terminus of said first object sequence, said pair of arm sequences forming a stem duplex having about 3 to about 35 complementary base pairs when said first object sequence is not hybridized to said first complement sequence; and wherein said at least one detectable label comprises an interactive label pair comprising a first label moiety conjugated to said 5' arm sequence and a second label moiety conjugated to said 3' arm sequence, said first and second label moieties interacting when said stem duplex is formed.

107. (Original) A probe according to claim 1, wherein said probe further comprises a pair of nucleic acid arm sequences consisting of a 5' arm sequence covalently linked to 5' terminus of said first complement sequence and a 3' arm sequence covalently linked to 3' terminus of said first complement sequence, said pair of arm sequences forming a stem duplex having about 3 to about 35 complementary base pairs when said first object sequence is not hybridized to said first complement sequence; and wherein said at least one detectable label comprises an interactive label pair comprising a first label moiety conjugated to said 5' arm sequence and a second label moiety conjugated to said 3' arm sequence, said first and second label moieties interacting when said stem duplex is formed.

108. (Original) A probe according to claim 1, wherein said probe further comprises two pairs of nucleic acid arm sequences, a first arm pair consisting of a first 5' arm sequence covalently linked to 5' terminus of said first object sequence and a first 3' arm sequence covalently linked to 3' terminus of said first object sequence and a second arm pair consisting of a second 5' arm sequence covalently linked to 5' terminus of said first complement sequence and a second 3' arm sequence covalently linked to 3' terminus of said first complement sequence, said first arm pair forming a first stem duplex having

about 3 to about 35 complementary base pairs and said second arm pair forming a second stem duplex having about 3 to about 35 complementary base pairs when said first object sequence is not hybridized to said first complement sequence; and wherein said at least one detectable label comprises two interactive label pairs, a first label pair comprising a first label moiety conjugated to said first 5' arm sequence and a second label moiety conjugated to said first 3' arm sequence and a second label pair comprising a third label moiety conjugated to said second 5' arm sequence and a fourth label moiety conjugated to said second 3' arm sequence, said first label pair interacting when said first stem duplex is formed and said second label pair interacting when said second stem duplex is formed.

109-116. (Canceled)

117. (Original) A probe according to claim 2, wherein said detectable label is a non-interactive label selected from the group consisting of fluorescers, luminescers, radioisotopes, enzymes, antibodies, antigens, and electrochemical labels.

118-130. (Canceled)

131. (Original) A probe according to claim 2, wherein said at least one detectable label comprises an interactive label pair comprising a first label moiety conjugated to said first object sequence and a second label moiety conjugated to said first complement sequence, said first and second label moieties interacting when said first hybridized duplex is formed.

132. (Original) A probe according to claim 2, wherein said at least one detectable label comprises an interactive label pair comprising a first label moiety conjugated to said second object sequence and a second label moiety conjugated to said second complement sequence, said first and second label moieties interacting when said second hybridized duplex is formed.

133. (Original) A probe according to claim 2, wherein said at least one detectable label comprises two different interactive label pairs, a first label pair comprising a first label moiety conjugated to said first object sequence and a second label moiety conjugated to said first complement sequence and a second label pair comprising a third label moiety conjugated to said second object sequence and a fourth label moiety conjugated to said second complement sequence, said first and second label moieties interacting when said first hybridized duplex is formed and said third and fourth label moieties interacting when said second hybridized duplex is formed.

134. (Original) A probe according to claim 133, wherein said first and third label moieties are a same moiety.

135. (Original) A probe according to any of claims 131-133, wherein said interactive label pair is a fluorescer and a quencher, wherein the interaction between said interactive label pair causes change in the fluorescence of said probe, said change in the fluorescence being selected from the group consisting of intensity decrease, lifetime decrease, polarization change, and wavelength change.

136-156. Canceled

157 (Original) A probe according to claim 2, wherein said probe further comprises a pair of nucleic acid arm sequences consisting of a 5' arm sequence covalently linked to 5' terminus of said first complement sequence and a 3' arm sequence covalently linked to 3' terminus of said first complement sequence, said pair of arm sequences forming a stem duplex having about 3 to about 35 complementary base pairs when said first complement sequence is not hybridized to said first object sequence; and wherein said at least one detectable label comprises an interactive label pair comprising a first label moiety conjugated to said 5' arm sequence and a second label moiety conjugated to said 3' arm sequence, said first and second label moieties interacting when said stem duplex is formed.

158. (Original) A probe according to claim 2, wherein said probe further comprises a pair of nucleic acid arm sequences consisting of a 5' arm sequence covalently linked to 5' terminus of said second complement sequence and a 3' arm sequence covalently linked to 3' terminus of said second complement sequence, said pair of arm sequences forming a stem duplex having about 3 to about 35 complementary base pairs when said second complement sequence is not hybridized to said second object sequence; and wherein said at least one detectable label comprises an interactive label pair comprising a first label moiety conjugated to said 5' arm sequence and a second label moiety conjugated to said 3' arm sequence, said first and second label moieties interacting when said stem duplex is formed.

159. (Original) A probe according to claim 2, wherein said probe further comprises two pairs of nucleic acid arm sequences, a first arm pair consisting of a first 5' arm sequence covalently linked to 5' terminus of said first complement sequence and a first 3' arm sequence covalently linked to 3' terminus of said first complement sequence and a second arm pair consisting of a second 5' arm sequence covalently linked to 5' terminus of said second complement sequence and a second 3' arm sequence covalently linked to 3' terminus of said second complement sequence, said first arm pair forming a first stem duplex having about 3 to about 35 complementary base pairs when said first complement sequence is not hybridized to said first object sequence and said second arm pair forming a second stem duplex having about 3 to about 35 complementary base pairs when said second complement sequence is not hybridized to said second object sequence; and wherein said at least one detectable label comprises two interactive label pairs, a first label pair comprising a first label moiety conjugated to said first 5' arm sequence and a second label moiety conjugated to said first 3' arm sequence and a second label pair consisting of a third label moiety conjugated to said second 5' arm sequence and a fourth label moiety conjugated to said second 3' arm sequence, said first label pair interacting when said first stem duplex is formed and said second label pair interacting when said second stem duplex is formed.

160-227. (Canceled)

228. (Previously Presented) A target detection system comprising at least one probe according to any one of claims 1, 2 and 5.

229. (Previously presented) The affinity probe of claim 5, wherein the probe ligand is biotin.

230. (Canceled)

231. (Previously Presented) The probe according to any of claims 1 and 2, wherein the target agent has a size between about 1nm to a few micrometers.

232. (Previously Presented) The probe according to any of claims 1 and 2, wherein the coupling agent has a length of between about 1nm to about 100nm.

233. (Canceled)

234. (Previously Presented) The affinity probe according to claim 5, wherein the receptor agent has a size between about 1nm to a few micrometers.

235. (Previously Presented) The affinity probe according to claim 5, wherein the coupling agent has a length of between about 1nm to about 100nm.

236. (Currently amended) A probe comprising as operably linked components:

- a) a first pair of nucleic acid sequences consisting of a first object sequence and a first complement sequence, said first object and first complement sequences each independently having about 3 to about 150 nucleotides, being substantially complementary to each other, and forming a first hybridized duplex;
- b) at least one recognition element conjugated to at least one of said first object and first complement sequences through a coupling element, said recognition

element specifically interacting with at least one target agent, said coupling element being essentially the same size as or shorter than the size of said target agent; and

- c) at least one detectable label, said detectable label producing a characteristic signal whose level is a function of the amount of said first hybridized duplex, wherein said recognition element is conjugated through said coupling element to a location at least one nucleotide inside the first hybridized duplex region of said first object or first complement sequence so that said recognition element is branched out from said first hybridized duplex; and

wherein in the presence of said target agent, said interaction of said target agent with said recognition element alters the amount of said first hybridized duplex compared to that in the absence of said target agent, altering said characteristic signal-

and further wherein said recognition element is selected from the group consisting of chemical ligands, biochemical ligands, antigens, antibodies, antibody fragments, enzymes, substrates of enzymes, inhibitors of enzymes, hormones, antibiotics, narcotics, toxins, polypeptides, proteins, protein fragments, targeting sequences, transit peptides, glycoproteins, lipids, phospholipids, polysaccharides, carbohydrates, and peptide nucleic acids.

237. (Currently amended) An affinity probe, comprising as operably linked components:

- a) a first pair of nucleic acid sequences consisting of a first object sequence and a first complement sequence, said first object and first complement sequences each independently having about 3 to about 150 nucleotides, being substantially complementary to each other, and forming a first hybridized duplex;
- b) at least one probe ligand conjugated to at least one of said first object and first complement sequences through a coupling element, said probe ligand specifically interacting with at least one receptor agent, said coupling element being essentially the same size or shorter than the size of said receptor agent; and

c) at least one detectable label, said detectable label producing a characteristic signal whose level of function of the amount of said first hybridized duplex, wherein said probe ligand is conjugated through said coupling element to a location at least one nucleotide inside the first hybridized duplex region of said first object or first complement sequence so that said probe ligand is branched out from said first hybridized duplex; and

wherein in the presence of said receptor agent, said interaction of said receptor agent with said probe ligand alters the amount of said first hybridized duplex compared to that in the absence of said receptor agent, altering said characteristic signal; and further wherein said receptor agent specifically binds to said probe ligand-

and further wherein said probe ligand is selected from the group consisting of chemical ligands, biochemical ligands, antigens, antibodies, antibody fragments, enzymes, substrates of enzymes, inhibitors of enzymes, hormones, antibiotics, narcotics, toxins, polypeptides, proteins, protein fragments, targeting sequences, transit peptides, glycoproteins, lipids, phospholipids, polysaccharides, carbohydrates, and peptide nucleic acids.